Check List the journal of biodiversity data

LISTS OF SPECIES

Check List 11(4): 1676, 13 June 2015 doi: http://dx.doi.org/10.15560/11.4.1676
ISSN 1809-127X

Hematophagous biting midges (Diptera: Ceratopogonidae) from Tefé municipality, Amazonas state, Brazil

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Abstract: Some species of biting midges are vectors of pathogens that cause disease in vertebrates, including humans. The aim of this study was to survey the biting midge fauna in Tefé, Amazonas, Brazil. *Culicoides* were collected using HP light traps during January, February, and April 2013. Midges collected included one species from the genus *Lectoconops* that bites humans. A total of 248 *Culicoides* individuals were collected, representing 19 species from two subgenera (*Haematomyidium* and *Hoffmania*) and four informal species groups (*carpenteri*, *leoni, fluvialis*, and *reticulatus*). Twelve individuals of *L. brasiliensis* were also collected, and this is the first record of *L. brasiliensis* in Amazonas. Three species of *Culicoides* were also recorded for the first time in Amazonas, including *Culicoides aitkeni*, *C. glabellus* and *C. ocumarensis*. The most abundant species were *C. hylas* (81 individuals), and *C. foxi* (27). The *Culicoides* fauna in Tefé is diverse, and proven vectors such as *C. paraensis* and *C. insignis* were found.

Key words: biting midges, diversity, forest environment, peridomicile environment, Middle Solimões, Amazonia

INTRODUCTION

Some species of biting midges are significant vectors for several pathogens, including the viruses that cause Oropouche fever in humans and Bluetongue in domestic and wild animals (Mellor et al. 2000; Borkent and Spinelli 2007; Nunes et al. 2007). The most significant of these species is *Culicoides* Latreille, 1809. The genera *Culicoides* and *Leptoconops* Skuse, 1889 also transmit *Mansonella* Faust, 1929, which causes Mansonelliasis in humans. The bites of these insects cause skin irritation in humans and animals (Borkent 2005; Borkent and Spinelli 2007).

Culicoides are distributed widely throughout the world, with 273 species reported in the Neotropical region (Borkent 2012). Veras (2001) listed 82 species from the Brazilian Amazon region. Recently, 12 more species were described by Spinelli et al. (2007), Felippe-Bauer et al. (2009, 2010, 2013), Trindade and Felippe-Bauer (2011) and Santarém et al. (2014) from the states of Amazonas, Pará and Roraima. Considering that the distribution of *Culicoides reticulatus* Lutz, 1913 is now restricted to Bahia and Pernambuco states (Santarém et al. 2014), we consider 93 species known for the Brazilian Amazon.

In the Neotropical region, 12 species of the genus *Leptoconops* are distributed within four subgenera: *Brachyconops* Wirth and Atchley, 1973, *Holoconops* Kiefer, 1908, *Leptoconops*, and *Megaconops* Wirth and Atchlei, 1973 (Borkent and Spinelli 2000, 2007). In Brazil, only two species have been recorded: *L.* (*H.*) *knowtoni* Clastrier and Wirth, 1978 from Santa Catarina state; and *L.* (*L.*) *brasiliensis* Lutz, 1913 from Amazonas state; however, the results of Borkent and Spinelli (2000) were corrected by Trindade and Gorayeb (2005), who restricted the distribution of *L. brasiliensis* to Pará state. Pessoa et al. (2012) have found *L. brasiliensis* in Acre state, on the triple border of Brazil, Peru, and Bolivia, and they speculated that the species is distributed widely throughout the Amazon basin.

Recent studies have shown that Mansonelliasis is endemic in the Middle Solimões basin, and that infection prevalence is high in humans that live in the riverine communities of Tefé municipality (Medeiros et al. 2014a, 2014b). These communities are situated in "várzea" environments (lowland Amazonian forest that floods in the rainy season), and "terra firme" environments (upland Amazonian forest that never floods); these two environments comprise most of the Amazonas state landscape. However, until now,

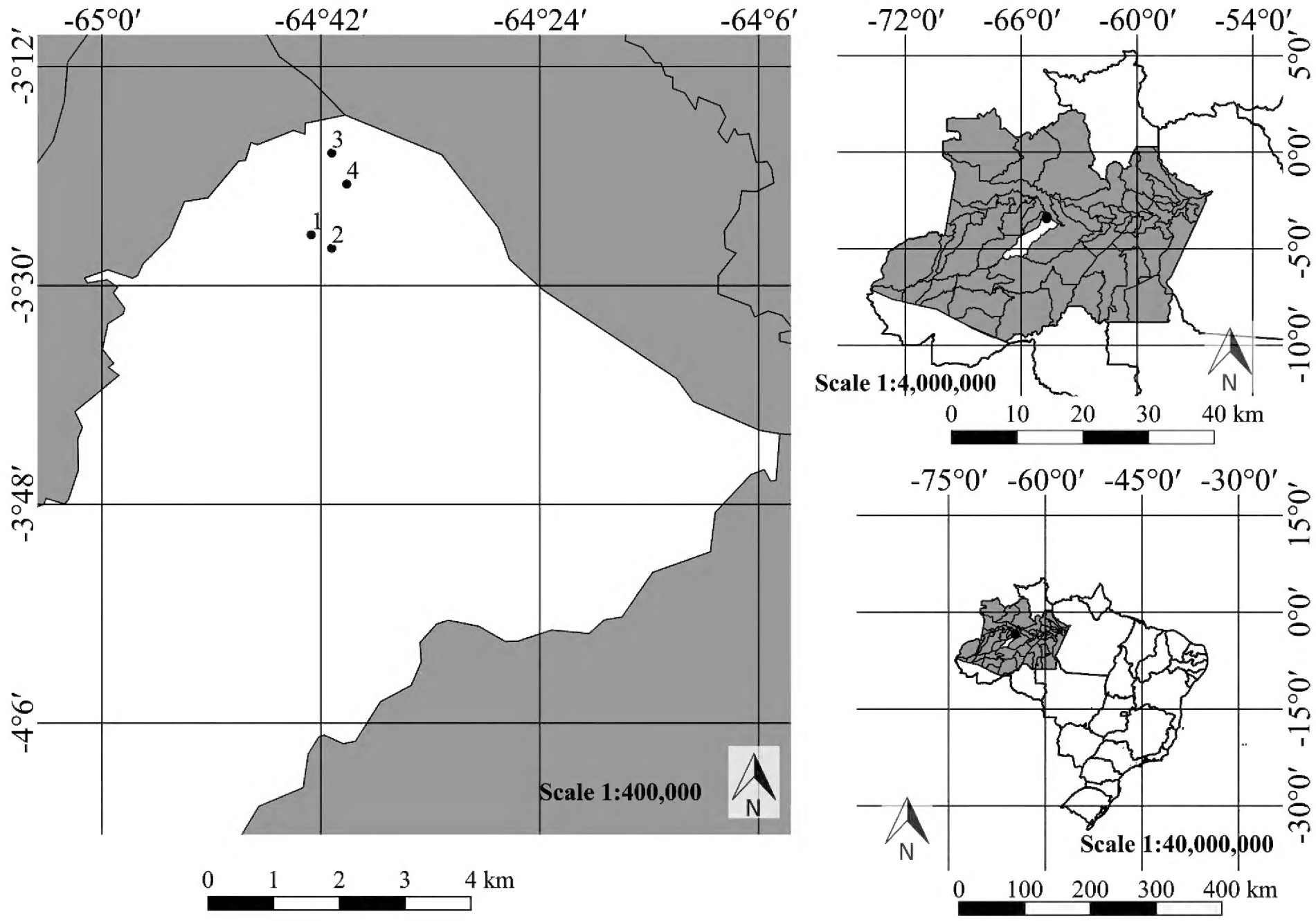

Figure 1. Map of study area. Location of biting midges collection areas in January, February and April 2013 in Tefé municipality, Amazonas, Brazil. Numbers correspond to locales: (1) km 03, (2) km 08, (3) Nossa Senhora do Perpetuo Socorro community and (4) Porto Vale community.

surveys of hematophagous biting midges fauna have not been conducted in this region. The aim of this study was to survey the biting midges fauna of the genera *Culicoides* and *Leptoconops* and identify differences in faunal composition between "várzea" and "terra firme" environments along the Solimões River.

MATERIALS AND METHODS

Study area

Tefé municipality (03°21′05″ S, 064°42′53″ W) is located in the middle of Amazonas state, Brazil. It is one of ten municipalities that comprise the Middle Solimões region (Figure 1). It is 23,704.488 km² in area, and has a population of 61,453 inhabitants (IBGE 2014). The climate is classified as Afi in the Köppen classification scheme: tropical rainforest with no dry season (minimum rainfall of 60 mm per month), and no cold season (monthly temperatures averaging above 18°C with annual temperatures oscillating by no more than 5°C) (INMET 2013). The main vegetation is comprised of dense ombrophylous forest lowlands (rolling lowlands that do not flood and have emerging canopy trees) and alluvial ombrophylous forests (which occur along water courses and are subject to annual white water flooding; they can have open canopies, uniform canopies, or emerging canopy trees) (Vivona et al. 2003).

Biting midges were collected in forest and peridomicile areas near small farms in both "terra firme" (upland) and "várzea" (lowland) environments. The entomological surveys were conducted in the following localities: EMADE road (km 03 and km 08), Nossa Senhora do Perpétuo Socorro community, and Porto Vale community (Figure 1). Collections were made using HP light traps that were set for eight consecutive nights per month (6:00–7:00 h) during January, February, and April 2013. In June 2012, Medeiros et al. (2014b) conducted an epidemiological study of Mansonelliasis in Tefé municipality; they collected some hematophagous insects in the margins of the Solimões River near "várzea" environments, and they collected some diurnal biting midges.

Data collection

Following each capture event, biting midges were preserved in 90% alcohol and taken to Laboratório de Ecologia e Doenças Transmissíveis da Amazônia (EDTA/Fiocruz-Amazônia) in Manaus Municipality, Amazonas state. The specimens were slide-mounted in phenol-balsam in the manner described by Wirth and Marston (1968). Identification was performed using a variety of identification keys, including those of Spinelli et al. (1993, 2005), Veras (2001), Felippe-Bauer et al. (2009).

Descriptive statistical analysis was performed to identify differences between environments; data from forest and peridomicile areas was compared using BioEstat version 5.2 to apply the Mann-Whitney test with a significance level of 0.05.

RESULTS

A total of 248 *Culicoides* individuals were collected, including 14 males (5.6%) and 234 females (94.4%), representing a total of 19 species from two subgenera (*Haematomyidium* Goeldi, 1905 and *Hoffmania* Fox, 1948) and four informal species groups (*carpenteri*, *leoni*, *fluvialis*,and *reticulatus*); some individuals were considered morphotypes (*n* = 32): *Culicoides* sp. 1 of subgenus *Haematomyidium*; *C.* sp. 2, *C.* sp. 3, and *C.* sp. 4 of the subgenus *Hoffmania*; and *C.* sp. 5, *C.* sp. 6, and *C.* sp. 7 of an unplaced subgenus from the *reticulatus* group. Three species were reported for the first time in Amazonas state: *C. aitkeni* Wirth and Blanton, 1968, *C. glabellus* Wirth and Blanton, 1956, and *C. ocumarensis* Ortíz, 1950. The most abundant species/morphotypes were *C. hylas* Macfie, 1940 (*n* = 81, 32.7%), *C. foxi* Ortíz, 1950 (*n* = 27, 10.9%), *C.* sp. 7 (*n* = 23, 9.3%), *C. pseudodiabolicus* Barbosa, 1946 (*n* = 22, 8.9%), *C. paucienfuscatus* Barbosa, 1947 (*n* = 22, 8.9%) and *C. insignis* Lutz, 1913 (*n* = 20, 8.1%) (Table 1). From the genus *Leptoconops*, 12 individuals belonging to *L. brasiliensis* were collected, but as this species was collected at a different time using a different method, it was not included in Table 1.

The "terra firme" environments exhibited high abundance and low species richness (147 individuals and 15 species) in comparison with lowland "várzea" environments (101 individuals and 17 species). The most abundant species in "terra firme" environments were *C. hylas* (*n* = 79, 31.8%), *C. foxi* (*n* = 21, 8.4%), and *C. paraignacioi* Spinelli, 1993 (*n* = 10, 4.0%). The species unique to this environment included: *C. aitkeni*, *C. benarrochi* Ortíz and Mirsa, 1952, *C. coutinhoi* Barretto, 1944, *C. filariferus* Hoffman, 1939, *C. glabrior* Macfie, 1940, *C. ocumarensis* and *C. paraignacioi*. The most abundant species/morphotypes in "várzea" environments were *C.* sp. 7 (*n* = 23, 9.2%), *C. paucienfuscatus* (*n* = 22, 8.8%), and *C. insignis* (*n* = 20, 8.1%). The species unique to this environment include *C. belemensis* Wirth and Blanton, 1973, *C. debilipalpis* Lutz, 1913, *C. insignis*, *C. leopoldoi* Ortíz, 1951, *C. paraensis* (Goeldi, 1905) and *C. paucienfuscatus* Barbosa, 1947. Forests exhibited higher abundance than peridomicile areas (Mann-Whitney U = 36 P = 0.03).

DISCUSSION

The 19 species of *Culicoides* found in Tefé (and the seven morphotypes) represent approximately 25% of the *Culicoides* fauna in the Brazilian Amazon. The diversity of *Culicoides* fauna in the Brazilian Amazon has likely been underestimated because too few *Culicoides* studies in this region. The seven morphotypes documented here may represent new species. In a recent revision of the *reticulatus* species group, five new species were described in the Brazilian Amazon (Santarém et al. 2014), which demonstrates that our knowledge of *Culicoides* fauna remains incomplete. The low density of males in the catch is most likely the result of copulation behavior (Castellon et al. 1990) that usually occurs near oviposition sites and food sources.

Culicoides aitkeni, *C. glabellus* and *C. ocumarensis* were recorded for the first time in Amazonas state. *Culicoides aitkeni* is prevalent in Trinidad and Tobago and Brazil, where it occurs in Pará state (Felippe-Bauer et al. 2009). *Culicoides glabellus* is prevalent between Honduras and Ecuador, in Trinidad and Tobago, and in Brazil, where it occurs in Bahia state (Borkent and Spinelli 2007). *Culicoides ocumarensis* is prevalent throughout the Americas, from the United States to Argentina, and it has been recently isolated in Peru and Brazil, where it occurs in the states of Pará and Rondônia (Borkent and Spinelli 2007; Felippe-Bauer et al. 2008).

Lutz (1913) described *L. brasiliensis* (as *Tersethes brasiliensis*) from the lower Tocantins River, but he did not identify the state, which was Pará. Borkent and Spinelli (2000) used the type locality to establish the presence of *L. brasiliensis* in Amazonas state, but Trindade and Gorayeb (2005) corrected this result by restricting *L. brasiliensis* to Pará state. Pessoa et al. (2012) recorded *L. brasiliensis* in Acre state in the Southeastern Amazon. This means that our collections in Tefé municipality are the first confirmed finds of *L. brasiliensis* in Amazonas state.

Culicoides species diversity was higher in "várzea" environments than it was in "terra firme" environments. Studies in Amazonas state have been conducted primarily in "terra firme" environments (Castellón and Ferreira 1991; Veras and Castellón 1998; Veras 2001; Silva and Bermúdez 2009). In "várzea" environments, *Culicoides* fauna remains largely unknown. The fauna found in forests was richer and more abundant than fauna found in peridomicile areas, and peridomiciles in "terra firme" environments exhibited significantly less richness and abundance than all other environments.

This study identified *C. hylas* as the most abundant species in Tefé municipality, and this species has also been identified as the most abundant in other regions of Amazonas state, including Manaus and São Gabriel da Cachoeira (Veras and Castellón 1998; Silva and Bermúdez 2009), and *C. hylas* is not currently considered a species of medical importance. Of the species collected in this study, nine have anthropophilic habits: *C. benarrochi*, *C. debilipalpis*, *C. foxi*, *C. glabellus*, *C. insignis*, *C. leopoldoi*, *C. paraensis*, *C. paraignacioi*, *C. pseudodiabolicus* (Aitken et al. 1975; Bermúdez 1986; Castellón and Ferreira 1991;

Table 1. *Culicoides* collected with HP light traps in "várzea" and "terra firme" environments in Tefé municipality, Amazonas state, Brazil during the months of January, February, and April 2013.

Subgenus /Species Group	Species	"Várzea" F ♂	"Várzea" F ♀	"Várzea" P ♂	"Várzea" P ♀	"Terra Firme" F ♂	"Terra Firme" F ♀	"Terra Firme" P ♂	"Terra Firme" P ♀	"Várzea" Total	"Terra Firme" Total	Total	%
Haematomyidium	*Culicoides debilipalpis* Lutz, 1913	1	0	0	0	0	0	0	0	1	0	1	0.4
	Culicoides glabrior Macfie, 1940	0	0	0	0	3	5	0	0	0	8	8	3.2
Haematomyidium/ paraensis Group	*Culicoides paraensis* (Goeldi,1945)	1	1	0	0	0	0	0	0	2	0	2	0.8
	*Culicoides*sp. 1	0	0	0	0	0	0	0	1	0	1	1	0.4
Hoffmania/ gutatus Group	*Culicoides coutinhoi* Barretto, 1944	0	0	0	0	0	2	0	0	0	2	2	0.8
	Culicoides diabolicus Hoffman, 1925	0	0	0	1	0	2	0	0	1	2	3	1.2
	Culicoides filariferus Hoffman, 1939	0	0	0	0	0	2	0	0	0	2	2	0.8
	Culicoides foxi Ortíz, 1950	0	2	0	4	0	20	0	1	6	21	27	10.9
	Culicoides insignis Lutz,1913	0	0	0	20	0	0	0	0	20	0	20	8.1
	Culicoides ocumarensis Ortíz, 1950*	0	0	0	0	0	6	0	0	0	6	6	2.4
	Culicoides paraignacioi Spinelli, 1993	0	0	0	0	0	10	0	0	0	10	10	4.0
	Culicoides pseudodiabolicus Fox, 1946	0	8	0	5	0	9	0	0	13	9	22	8.9
	Culicoides sp. 2	0	1	0	0	0	0	0	0	1	0	1	0.4
	Culicoides sp. 3	0	0	0	0	0	3	0	0	0	3	3	1.2
	Culicoides sp. 4	0	0	0	1	0	0	0	0	1	0	1	0.4
Hoffmania/ hylas Group	*Culicoides aitkeni* Wirth and Blanton, 1968*	0	0	0	0	0	1	0	0	0	1	1	0.4
	Culicoides hylas Macfie, 1940	0	2	0	0	8	71	0	0	2	79	81	32.7
	Culicoides verecundus Macfie, 1949	0	1	0	1	0	1	0	0	2	1	3	1.2
Unplaced/ *carpenteri* Group	*Culicoides belemensis* Wirth and Blanton, 1973	0	1	0	1	0	0	0	0	2	0	2	0.8
Unplaced/ *fluvialis* Group	*Culicoides leopoldoi* Ortíz, 1951	0	2	0	0	0	0	0	0	2	0	2	0.8
Unplaced/ *leoni* Group	*Culicoides benarrochi* Ortíz and Mirsa. 1952	0	0	0	0	0	1	0	0	0	1	1	0.4
	*Culicoides glabellus*Wirth and Blanton, 1956*	0	1	0	0	0	1	0	0	1	1	2	0.8
Unplaced/ *reticulatus* Group	*Culicoides paucienfuscatus* Barbosa, 1947	0	0	1	21	0	0	0	0	22	0	22	8.9
	Culicoides sp. 5	0	0	0	1	0	0	0	0	1	0	1	0.4
	Culicoides sp. 6	0	0	0	1	0	0	0	0	1	0	1	0.4
	Culicoides sp. 7	0	1	0	22	0	0	0	0	23	0	23	9.3
Total		**2**	**20**	**1**	**78**	**11**	**134**	**0**	**2**	**101**	**147**	**248**	**100**
%		**0.8**	**8.1**	**0.4**	**31.5**	**4.4**	**54.0**	**0.0**	**0.8**	**40.7**	**59.3**	**100**	

F: Forest, P: Peridomicile.*New records to Amazonas state.

Trindade and Gorayeb 2005, 2010; Costa et al. 2013). In the areas surveyed, *C. paraensis* and *C. insignis* were found in relatively low numbers; however, these species are considered to be of medical and veterinary importance, and it is possible for some species to be underrepresented in entomological studies. Low frequency can be the result of several factors including collection method, type of bait, environmental characteristics, and the presence of animals (Costa et al. 2013). Low frequency may also be related to diverse activity patterns which could cause diurnal and nocturnal species to be disproportionately represented (Koch and Axtel 1979; Hoch et al. 1990).

Culicoides paraensis has been proven to be a vector of Oropouche virus (Pinheiro et al. 1981; Borkent and Spinelli 2007). It is found throughout the Americas, from Argentina to the United States (Felippe-Bauer et al. 2003), and it is the most studied species of the New World *Culicoides*. High incidences of ORO virus have been reported in urban and wild areas in several states of the Brazilian Amazon. It is estimated that half a million cases have occurred in Brazil over the last 30 years (Borkent 2005; Teixeira Nunes et al. 2005; Azevedo et al. 2007). Outbreaks of Oropouche fever have also occurred in Trinidad and Tobago, Panamá, Peru, and Ecuador (Baisley et al. 1998; Borkent 2005; Azevedo et al. 2007; PAHO 2010).

Culicoides paraensis has been incriminated as a secondary vector of *Mansonella ozzardi* (Manson, 1897) (Romaña and Wygodzinsky 1950; Shelley and Coscarón 2001), and it has been frequently found in the Upper Rio Negro region of Amazonas state (Silva and Bermúdez 2009). *Mansonella ozzardi* is widely distributed throughout Amazonas state; instances have been recorded since the late 1940s (Deane 1949), and high levels of transmission and infection prevalence have been observed in the Middle Solimões basin (Martins et al. 2010; Medeiros et al. 2014a, 2014b).

Culicoides insignis is a proven vector of bluetongue virus (BTV) and is found throughout the Americas, from the United States to Argentina. In Brazil, BTV has spread through the southern parts of the North Region, with positive serological cases recorded in several states including Amazonas (Scolari et al. 2011; Dorneles et al. 2012). In the Amazon Basin, the occurrence of *L. brasiliensis* is probably widespread, and beside the effects of its bites, the medical importance in Brazil is unknown; however, the fact that *L. brasiliensis* has been collected in an area of endemic mansonelliasis, means that it should be studied further.

Conclusion

The hematophagous biting midges fauna in Tefé municipality was found to be diverse. Abundance was highest in upland environments, while species diversity was slightly higher in lowland environments. The fauna surveyed included species incriminated as vectors of Oropouche virus, *Mansonella* and bluetongue virus, which indicates that this region could be at risk. Further studies using different capture methods will be needed to improve our understanding of *Culicoides* and *Leptoconops* fauna in the Middle Solimões basin.

ACKNOWLEDGEMENTS

The authors would like to acknowledge: the financial support of FAPEAM, Grant Number 007/2009; the inhabitants of the Nossa Senhora do Perpétuo Socorro and Porto Vale communities; FAPEAM PAIC from whom JAF had Grant Number 062.01577/2014; and to Maria Luiza Felippe-Bauer for her invaluable corrections to, and suggestions for, the first draft of the manuscript.

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Authors' contribution statement: ESF participated in sample design, identified the insects, and co-wrote the text; AMPJ participated in sample design, collected insects, and co-wrote the text; JFA mounted the insects and co-wrote the article; FACP participated in sample design, collected insects, and co-wrote the text; JFM participated in sample design, collected insects, performed the data analysis, and co-wrote the text.

Received: 26 March 2015
Accepted: 2 June 2015
Academic editor: Jonas da Silva Döge